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Antifungal activity of a series of 1,2-benzisothiazol-3(2*H*)-one derivatives

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ABSTRACT

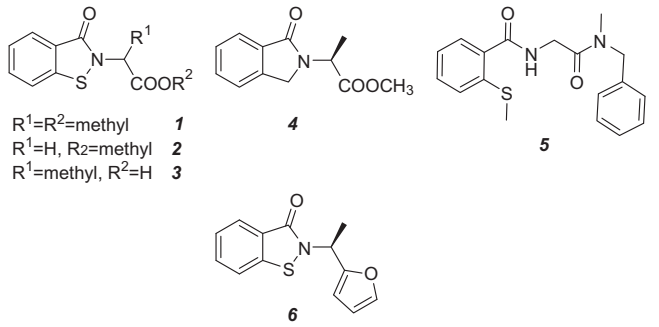
A series of broad-spectrum antifungal agents based on the 1,2-benzisothiazol-3(2*H*)-one scaffold is reported. Preliminary structure–activity relationship studies have established the importance of the presence of the heterocyclic ring, a methyl group, and a phenyl ring for optimal manifestation of antifungal activity.

1. Introduction

Invasive fungal infections are an emerging health problem and the leading cause of morbidity and mortality in immune-suppressed patients.[1] Current therapy for fungal infections involves the use of polyenes, azoles, echinocandins, allylamines, and antimetabolites.[2–4] These agents exert their antifungal effects through different mechanisms: polyenes, such as amphotericin B, compromise membrane integrity and alter membrane permeability by binding to fungal cell membrane sterols (ergosterol); azoles block the production of ergosterol, an essential membrane constituent, by inhibiting lanosterol C-14α-demethylase[5,6]; echinocandins arrest fungal cell wall synthesis by inhibiting 1,3-β-D-glucan synthase[7]; allylamines block the biosynthesis of fungal cell wall sterols through the inhibition of squalene epoxidase; and antimetabolites, such as 5-fluorocytosine, interfere with DNA and RNA synthesis. The use of these agents is plagued by toxicity, limited efficacy, and the emergence of resistance, consequently, there is a pressing need for new and effective antifungal therapeutics that are fungicidal, fungal-specific, and have a broad spectrum of activity and novel mechanisms of action.[8–11]

Screening of a subset of compounds from an in-house library of compounds resulted in the identification of compound **1** (Table 1) that exhibited noteworthy antifungal activity against *Candida* species, *Aspergillus fumigatus* and *Cryptococcus neoformans*. Based on this observation, structure–activity relationship (SAR) studies aimed at delineating the structural elements in compound **1** responsible for its antifungal activity and identifying drug-like antifungal compounds that display optimal potency, selectivity, pharmacokinetics and oral bioavailability, were undertaken.[12] We describe herein preliminary results related to the development of antifungal agents based on the 1,2-benzisothiazol-3(2*H*)-one scaffold (structure (I), Table 2).

Table 1



2. Chemistry

Compounds **7–25** were readily synthesized as shown in Schemes 1 and 2. The 1,2-benzisothiazol-3(2*H*)-one *N*-carboxymethyl intermediate **2a** (Scheme 1) was prepared by treating 1,2-benzisothiazol-3(2*H*)-one with sodium hydride in acetonitrile, followed by evaporation of the solvent and addition of trifluoroethanol and *t*-butyl bromoacetate. This protocol ensured near

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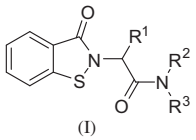
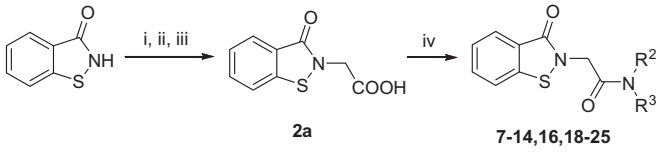
Table 2



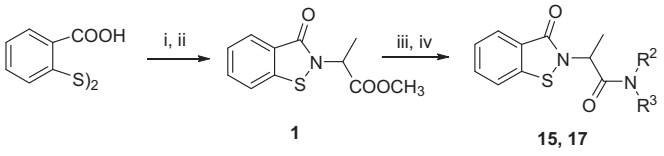
(I)

Compd	R^1	R^2	R^3	MIC_{50} (μg/mL)	
				C. albicans (CAF-2)	*C. glabrata* (RC-201)
7	H	H	Phenyl	6.4	6.4
8	H	H	(*o*-$COOCH_3$)phenyl	6.4	12.5
9	H	H	(*m*-$COOCH_3$)phenyl	12.5	25
10	H	Methyl	Phenyl	>50	>50
11	H	H	Benzyl	3.2	6.4
12	H	H	(*m*-OCH_3)benzyl	3.2	6.4
13	H	H	(*p*-OCH_3)benzyl	3.2	3.2
14	H	H	(*p*-F)benzyl	1.6	3.2
15[a]	Methyl	H	Benzyl	>50	>50
16	H	Methyl	Benzyl	1.6	1.6
17[a]	Methyl	Methyl	Benzyl	25	25
18	H	Ethyl	Benzyl	6.4	12.5
19	H	Ethyl	Ethyl	>50	>50
20	H	H	(*S*) $CH(CH_3)$ph	6.4	12.5
21	H	H	(*R*) $CH(CH_3)$ph	6.4	6.4
22	H	H	$(CH_2)_2$ph	>50	>50
23	H	H	1-Adamantyl	3.2	6.4
24	H	H	2-Thiazolyl	>50	>50
25	H	H	$(CH_2)_2$morpholino	>50	>50

[a] Racemate.



Scheme 1. General synthesis of compound (I). Reactions and conditions: (i) NAH/CH_3CN; (ii) $BrCH_2COO^tBu/CuF_3CH_2OH$; (iii) TFA; (iv) EDCI/HOBt/DMF then amine.



Scheme 2. Synthesis of compounds **15** and **17**. Reactions and conditions: (i) CDI/THF then (DL) Ala-OCH_3; (ii) Br_2/TEA; (iii) LiOH/aq THF; (iv) EDCI/HOBt/DMF then amine.

exclusive formation of the N-alkylated product which was treated with trifluoroacetic acid to yield the free carboxylic acid **2a**. Subsequent coupling with an array of structurally-diverse amines yielded the desired compounds **7–14**, **16**, **18–25** (structures shown in Table 2). Compounds **15** and **17** were prepared by refluxing dithiosalicylic acid with carbonyl diimidazole in dry tetrahydrofuran followed by the addition of triethylamine and (DL) alanine methyl ester hydrochloride (Scheme 2). The resulting diester was then treated with bromine followed by triethylamine to yield the 1,2-benzisothiazol-3(2*H*)-one-derived methyl ester which was hydrolyzed to the acid with LiOH in aqueous THF. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDCI)-mediated coupling with an array of amines yielded the final products.

Table 3

Fungus	MIC_{50} (μg/mL)	
	Compound **16**	Fluconazole
Candida albicans (CAF-2)	1.6	0.2
Candida glabrata[a]	1.6	3.2
Candida tropicalis[a]	1.6	16
Candida parapsilosis[a]	1.6	0.4
Candida lusitaniae[a]	1.6	3.2
Candida guillermondii[a]	1.6	6.4
Candida apicola[a]	1.6	0.2
Aspergillus fumigatus (AF-298)	12.5	16
Cryptococcus neoformans (H-99)	1.6	>64
Cryptococcus neoformans (JEC-21)	1.6	>64

[a] Clinical strains.

3. Biology

The synthesized compounds were initially screened against *C. albicans* (CAF-2) and *C. glabrata* (RC-201, clinical strain) and the results are shown in Table 2. The most active compound **16** was also screened against other species of *Candida*, *Aspergillus fumigatus* and *Cryptococcus neoformans* and found to exhibit antifungal activity (Table 3).

4. Results and discussion

The structure of the hit (compound **1**, Table 1) was probed using an iterative approach involving structural modifications and in vitro screening. The importance of the α-methyl group was quickly established by demonstrating that compound **2** is devoid of antifungal activity. The parent heterocycle, 1,2-benzisothiazol-3(2*H*)-one, also lacks antifungal activity, suggesting that the presence of the heterocyclic ring is necessary but not sufficient for the manifestation of antifungal activity. This is clearly evidenced by the lack of activity

by the corresponding isoindolin-1-one carboxymethyl methyl ester **4** (Table 1). This is further supported by the observation that compound **16** (Table 2) exhibits potent antifungal activity, while the corresponding acyclic compound **5** (Table 1) is inactive. The individual enantiomers of **1** were also synthesized and shown to be both active, however, the (L) enantiomer was more potent than the (D) enantiomer (MICs 1.6 and 3.2 μg/mL, respectively). Furthermore, replacement of the ester group by a furan ring yielded compound **6** (Table 1), which was also active. The lack of activity shown by compound **3** (Table 1) is likely due to poor permeability. These early observations attested to the importance of the heterocyclic ring and the α-methyl group (vide infra) for optimal interaction with the putative receptor whose identity has not been determined, as yet. It should be noted that the aforementioned findings are in accord with earlier literature reports regarding the biological activity, including antifungal[13] and antibacterial activity,[14] of suitably-substituted 1,2-benzisothiazol-3(2*H*)-one derivatives.[15]

The labile nature of the ester group necessitated its replacement by a more robust functionality. Thus, a series of amides was synthesized and then screened against *C. albicans* and *C. glabrata* (Table 2). Initial screening was done with two species of *Candida* to look for activity against the two most medically important species. Infections by *C. albicans* are most prevalent, while infections by *C. glabrata* have been increasing in prevalence over the past 10–15 years. Furthermore, *C. glabrata* has intrinsic drug resistance against azole drugs, which is a major problem in hospitals. The following inferences can be drawn by inspection of the results shown in Table 3: (a) multiple compounds derived from scaffold (I) have noteworthy antifungal activity against both *C. albicans* and *C. glabrata* (compounds **11–14**, **16**, **23**, Table 2); (b) antifungal activity is generally higher for compounds having an R^3 = benzyl (compounds **11–14**, Table 2) and even higher when R^2 = methyl and R^3 = benzyl (compound **16**, Table 2). The presence of an N-methyl group appears to impact favorably antifungal activity as the presence of an α-methyl group (vide supra). An added advantage of the N-methyl substituted compounds is the absence of a chiral center that is prone to the loss of stereochemical integrity; (c) replacement of the methyl group with an ethyl group (compound **18**) reduces activity, suggesting that R^2 engages in a favorable hydrophobic interaction in a cavity that can best accommodate a methyl group; (d) it is clearly evident that antifungal activity is sensitive to steric effects arising from R^2 and R^3 and that a benzyl group is preferred. In the latter case, replacement of the benzyl group by a heterocyclic aromatic (compound **24**) abolishes antifungal activity. Likewise, extending the aliphatic chain (compound **22**) or extending the aliphatic chain and replacing the phenyl ring with a morpholine ring (compound **25)** also abolishes antifungal activity; (e) interestingly, when R^3 is the highly hydrophobic adamantyl group, activity is regained. Taken together, these observations suggest that the interaction of (I) with its receptor involves a specific hydrophobic interaction with the methyl group and, possibly, a π–π or cation–π interaction with the phenyl ring. The hydrophobic interactions were further probed using chiral compounds **20** and **21** (Table 2), however, no significant effect on antifungal activity was observed (compare compounds **20–21** and compound **11**, Table 2)**.**

The antifungal activity of compound **16** was investigated against a panel of fungi of clinical relevance. Compound **16** exhibited a broad range of activity against *Cryptococcus*, *Candida* and *Aspergillus* (Table 3). This is a significant finding since most existing antifungal agents with these properties have resistance or toxicity issues. This compound showed against all strains tested with minimum inhibitory concentrations (MIC-50) ranging between 0.8 and 12.5 μg/mL. The MIC-50 values of compound **16** are shown in comparison to Fluconazole (Table 3) to highlight some important differences. The MIC values were higher for compound **16** compared to the azole drugs against *Candida albicans*. However, compound **16** has better in vitro activity against non-albicans *Candida* (NAC) species, *Aspergillus fumigatus* and *Cryptococcus neoformans*. It is known that the NAC species have increased in incidence over the past decade and contribute to the trend of azole resistance in hospitals.[13] It is also seen that MIC values of azole drugs vary considerably between species and organisms. In the case of compound **16**, the MIC-50 values remain relatively unchanged (0.8–3.2 μg/ml) except in the case of *Aspergillus fumigatus* where the in vitro activity is still better than that of Fluconazole (12.5 vs. 16 μg/ml). Thus, it is evident from the results shown in Table 3 that compound **16** displays superior antifungal activity against the panel of fungi shown in Table 3.

The cell cytotoxicity (CC-50) is the concentration of compound that inhibits growth of 50% of the cell line used at the defined time point. The CC-50 values of compound **16** were 62.2, 25.7 and 23.2 μg/mL for the HepG2 cell line at 24, 48 and 72 h for the neutral red assays. The CC-50 values were 15–30 times the MIC-50 concentration for compound **16**. Similar results were seen in the MTT assay experiments in both the HepG2 and Huh7 cell lines.

In summary, a series of antifungal agents based on the 1,2-benzisothiazol-3(2*H*)-one scaffold has been reported. The results of ongoing mechanistic and hit-to-lead optimization studies will be reported in due course.

5. Experimental section

General: The ^1H and ^{13}C NMR spectra were recorded on a Varian XL-300 or XL-400 NMR spectrometer. Melting points were determined on a Mel-Temp apparatus and were uncorrected. HRMS were performed at the University of Kansas Mass Spectrometry Lab. Reagents and solvents were purchased from various chemical suppliers (Aldrich, Acros Organics, TCI America, and Bachem). Silica gel (230–450 mesh) used for flash chromatography was purchased from Sorbent Technologies (Atlanta, GA). Thin layer chromatography was performed using Analtech silica gel plates. The TLC plates were visualized using iodine and/or UV light. All compounds were homogeneous by TLC and had >95% purity.

5.1. Chemistry

5.1.1. Methyl 2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)propanoate (1)[13]

To a solution of dithiosalicylic acid (1.53 g; 5 mmol) in 10 mL THF and 6 mL DMF was added dropwise a suspension of carbonyl diimidazole (1.70 g; 10 mmol) in 10 mL THF. The reaction mixture was stirred at room temperature for 20 min and refluxed for 20 min. After the reaction was cooled to room temperature, (DL) alanine methyl ester hydrochloride (1.40 g; 10 mmol) and triethylamine (1.5 mL; 12 mmol) were added. The resulting reaction mixture was stirred at room temperature for 2 days. The solvent was removed and the residue was taken up in ethyl acetate (50 mL) and water (20 mL). The layers were separated and the organic layer was washed with 5% HCl (3 × 30 mL), saturated $NaHCO_3$ (3 × 30 mL) and brine (30 mL). The organic layer was dried over anhydrous sodium sulfate. The drying agent was filtered off and the solvent evaporated, leaving an intermediate (1.8 g; 76% yield) which was used directly in the next step. To a chilled solution of this intermediate (1.8 g; 3.78 mmol) in 25 mL methylene chloride was added bromine (0.60 g; 3.78 mmol). The reaction mixture was stirred for 30 min and then treated with triethylamine (0.77 g; 7.5 mmol). The reaction mixture was allowed to warm to room temperature and then refluxed for 30 min. The solution was cooled to room temperature and washed with brine (2 × 15 mL). The organic layer was dried over anhydrous sodium

sulfate, filtered, and the solvent was evaporated under vacuum, leaving compound **1** as a yellow oil (1.8 g; 100% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70 (d, J = 7.8 Hz, 3H), 3.77 (s, 3H), 5.50 (q, J = 8.2 Hz, 1H), 7.42 (t, J = 7.2 Hz, 1H), 7.56–7.66 (m, 2H), 8.09 (d, J = 8.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 16.89, 51.18, 66.71, 125.64, 127.43, 128.25, 132.081, 141.041, 164.66, 171.83.

5.1.2. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetic acid (2a)

A solution of 1,2-benzisothiazol-3(2*H*)-one (15.1 g; 100 mmol) in dry acetonitrile (120 mL) cooled in an ice-bath was treated with 60% w/w sodium hydride (4.8 g; 120 mmol). After the mixture was stirred for 15 min in the ice-bath, the solvent was removed under vacuum. The residue was again placed in an ice-bath and 2,2,2-trifluoroethanol (150 mL) was added, followed by *t*-butyl bromoacetate (19.8 g; 100 mmol). The ice-bath was removed and the reaction mixture was refluxed for 1 h. The solvent was removed under vacuum and ethyl acetate (200 mL) was added. The organic phase was washed with 5% aqueous HCl (2 × 75 mL), 5% aqueous $NaHCO_3$ (75 mL), brine (75 mL) and then dried over anhydrous Na_2SO_4. The drying agent was filtered off and the solvent were evaporated under vacuum, yielding the *t*-butyl ester as a yellow oil (20.5 g; 77% yield) which was used in the next step without further purification. A solution of the above ester (20.5 g; 77 mmol) in dry CH_2Cl_2 (200 mL) was treated with trifluoroacetic acid (TFA, 260 mL) for 3 h at room temperature and the solvent was removed under vacuum. The residue was washed with ethyl ether (3 × 75 mL). Residual ethyl ether was removed under vacuum to yield the desired product **2a** as a white solid (11.2 g; 69% yield), mp 229–230 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.57 (s, 2H), 7.40–8.12 (m, 4H).

5.1.3. Methyl 2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetate (2)[15]

Compound **2** was prepared using a similar procedure as that used in the synthesis of the corresponding *t*-butyl ester (described above) by using methyl bromoacetate as the alkylating agent. White solid (250 mg; 11% yield), mp 82–83 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.75 (s, 3H), 4.62 (s, 2H), 7.40 (t, J = 7.9 Hz, 1H), 7.52–7.63 (m, 2H), 8.03 (d, J = 7.5 Hz, 1H).

5.1.4. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)propanoic acid (3)[13]

Compound **1** (2.37 g; 10 mmol) was dissolved in 15 mL 1, 4-dioxane and treated with 20 mL 1 M LiOH at 0 °C for 1 h. The solution was neutralized with 10% HCl and the dioxane was evaporated off. The pH of the aqueous residue was adjusted to 1, forming a precipitate which was collected using vacuum filtration and washed with diethyl ether to give pure compound **3** as a gray solid (1.73 g; 77% yield), mp 201–202 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.57 (d, J = 6.0 Hz, 3H), 5.14 (q, J = 6.5 Hz, 1H), 7.45 (t, J = 6.2 Hz, 1H), 7.70 (t, J = 6.0 Hz, 1H), 7.88 (d, J = 6.5 Hz, 1H), 7.97 (d, J = 6.5 Hz, 1H).

5.1.5. (*S*)-2-(1-(Furan-2-yl)ethyl)benzo[*d*]isothiazol-3(2*H*)-one (6)

To a stirred solution of 1,2-benzisothiazol-3(2*H*)-one (1.51 g; 10 mmol) and D-1-(2-furyl)ethanol (1.12 g; 10 mmol) in dry THF (25 mL) was added triphenylphosphine (5.24 g; 20 mmol), followed by the dropwise addition of a solution of diethylazodicarboxylate (DEAD) (97%; 3.60 g; 20 mmol) in 10 mL THF. The reaction mixture was stirred at room temperature for 4 h. The solvent was removed and the crude product was purified using flash chromatography (silica gel/hexane/ethyl acetate) to give pure compound **6** as a yellow solid (0.50 g; 20 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.74 (d, J = 6.2 Hz, 3H), 6.04 (q, J = 6.5 Hz, 1H), 6.37–6.41 (m, 2H), 7.35–7.58 (m, 4H), 8.02–8.06 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.32, 46.80, 108.36, 110.47, 120.62, 125.15, 125.58, 126.87, 131.94, 140.67, 143.04, 153.46, 164.98.

5.2. Representative synthesis for acid-amine coupling

5.2.1. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-phenylacetamide (7)

A solution of compound **2a** (0.5 g; 2.39 mmol) in dry DMF (5 mL) was treated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) (0.5 g; 2.63 mmol) with stirring. After stirring for 15 min, aniline (0.23 g; 2.39 mmol) was added and the reaction mixture was stirred overnight. The solvent was removed under vacuum, and the residue was taken up with ethyl acetate (50 mL). The organic solution was washed with 5% HCl (30 mL), 5% $NaHCO_3$ (30 mL), brine (30 mL) and then dried over anhydrous sodium sulfate. Removal of the solvent left a solid residue which was washed with diethyl ether (3 × 20 mL), leaving a white solid (0.20 g; 29% yield), mp 204–206 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.71 (s, 2H), 7.0–8.0 (m, 9H), 9.0 (t, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.11, 123.26, 123.40, 123.56, 125.40, 125.65, 125.91, 128.79, 128.86, 132.02, 137.21, 138.605, 141.48, 165.41, 167.50. HRMS (ESI): calcd for $C_{15}H_{13}N_2O_2S$ (M+H) 285.0698; found 285.0673.

5.2.2. Methyl 2-(2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamido)benzoate (8)

White solid (25% yield), mp 157–159 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.7 (s, 3H), 4.75 (s, 2H), 7.2–8.5 (m, 9H), 10.8 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.22, 52.42, 116.29, 118.23, 120.23, 125.73, 128.66, 131.66, 132.14, 134.03, 134.46, 137.33, 138.71, 139.79, 141.53, 168.32, 169.36. HRMS (ESI): calcd for $C_{17}H_{15}N_2O_4S$ (M+H) 343.0753; found 343.0737.

5.2.3. Methyl 3-(2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamido)benzoate (9)

White solid (40% yield), mp 219–220 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.82 (s, 3H), 4.85 (s, 2H), 7.43–8.35 (m, 8H), 10.9 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 46.16, 52.24, 119.64, 121.89, 123.41, 123.63, 124.13, 125.41, 125.66, 129.34, 130.16, 132.02, 139.07, 141.51, 165.08, 165.86, 166.03. HRMS (ESI): calcd for $C_{17}H_{15}N_2O_4S$ (M+H) 343.0753; found 343.0760.

5.2.4. *N*-Methyl-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-phenylacetamide (10)

Yellow solid (11% yield), mp 114–115 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.31 (s, 3H), 4.37 (s, 2H), 7.32–7.61 (m, 8H), 8.00 (d, J = 8.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 37.15, 44.82, 121.70, 123.25, 125.55, 126.17, 126.81, 127.38, 128.67, 129.97, 130.99, 131.94, 134.12, 141.59, 164.73, 165.90. HRMS (ESI): calcd for $C_{16}H_{14}N_2O_2S$ (M+Na) 321.0674; found 321.0683.

5.2.5. *N*-Benzyl-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (11)

White solid (30% yield), mp 185–187 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.29 (d, 2H), 4.55 (s, 2H), 7.2–8.0 (m, 9H), 8.7 (t, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.21, 45.61, 121.78, 123.54, 124.13, 125.35, 125.67, 126.87, 127.26, 127.38, 128.31, 131.93, 139.06, 141.42, 164.88, 166.69. HRMS (ESI): calcd for $C_{16}H_{15}N_2O_2S$ (M+H) 299.0854; found 299.0858.

5.2.6. *N*-(3-Methoxybenzyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (12)

White solid (36% yield), mp 149–150 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.77 (s, 3H), 4.29 (d, 2H), 4.55 (s, 2H), 6.8–8.0 (m, 9H), 8.7 (t, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 42.12, 45.65, 55.01, 112.33, 112.75, 119.35, 121.80, 123.56, 125.36, 125.65, 129.36, 131.93, 140.68, 141.43, 159.33, 164.89, 166.71. HRMS (ESI): calcd for $C_{17}H_{16}N_2O_3S$ (M+Na) 351.0779; found 351.0769.

5.2.7. *N*-(4-Methoxybenzyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (13)

Yellow solid (24% yield), mp 166–168 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.68 (s, 3H), 4.39 (d, J = 5.4 Hz, 2H), 4.55 (s, 2H), 6.56 (s, 1H), 6.8 (d, J = 9.1 Hz, 2H), 7.19 (d, J = 9.1 Hz, 2H), 7.42 (t, J = 7.8 Hz, 1H), 7.65 (m, J = 7.8 Hz, 2H), 8.01(d, J = 8.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 43.39, 47.66, 55.43, 114.21, 120.59, 123.45, 125.89, 126.70, 126.92, 129.25, 132.55, 141.25, 159.16, 166.14, 167.00. HRMS (ESI): calcd for $C_{17}H_{16}N_2O_3S$ (M+Na) 351.0779; found 351.0779.

5.2.8. *N*-(4-Fluorobenzyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (14)

Yellow solid (3% yield), mp 191–192 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.4 (d, 2H), 4.55 (s, 2H), 6.65 (s, 1H), 6.95 (t, 2H), 7.2 (m, 2H), 7.42 (t, 1H), 7.55–7.66 (m, 2H), 8.05 (d, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 43.06, 47.83, 115.59, 115.80, 120.65, 126.01, 126.93, 129.50, 129.58, 132.69, 141.17, 163.55, 166.22, 167.17. HRMS (ESI): calcd for $C_{16}H_{14}FN_2O_2S$ (M+H) 317.0760; found 317.0753.

5.2.9. (*RS*)-*N*-Benzyl-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)propanamide (15)

White solid (24% yield), mp 148–149 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.66 (d, J = 7.6 Hz, 3H), 4.37 (dd, J = 13.6, 5.3 Hz, 1H), 4.49 (dd, J = 13.6, 5.3 Hz, 1H), 5.43 (q, J = 6.4 Hz, 1H), 7.05 (s, 1H), 7.19–7.32 (m, 5H), 7.36 (t, J = 7.0 Hz, 1H), 7.55–7.64 (m, 2H), 7.91 (d, J = 6.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.84, 43.81, 52.59, 120.64, 124.24, 125.80, 126.77, 127.64, 127.74, 128.91, 132.35, 137.93, 141.33, 166.07, 169.77. HRMS (ESI): calcd for $C_{17}H_{16}N_2O_2S$ (M+Na) 335.0830; found 335.0824.

5.2.10. *N*-Benzyl-*N*-methyl-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (16)

White solid (34% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.00 (s, 3H), 4.60–4.77 (m, 4H), 7.19–7.42 (m, 6H), 7.49–7.63 (m, 2H), 7.96–8.06 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 33.87, 44.52, 50.47, 121.73, 125.30, 125.62, 126.83, 127.15, 127.57, 128.48, 128.77, 137.28, 141.46, 165.08, 166.44. HRMS (ESI): calcd for $C_{17}H_{17}N_2O_2S$ (M+H) 313.1011; found 313.1013.

5.2.11. (*RS*)-*N*-Benzyl-*N*-methyl-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)propanamide (17)

Yellow oil (20% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.53 (dd, J = 22.1, 3.7 Hz, 3H), 3.05 (d, J = 14.7 Hz, 3H), 4.5–4.7 (m, 2H), 5.9 (m, 1H), 7.18–7.5 (m, 7H), 7.59–7.64 (m, 2H), 7.9–8.2 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.07, 34.94, 48.58, 51.59, 120.47, 120.57, 125.48, 126.42, 126.74, 127.26, 127.81, 128.27, 128.89, 129.00, 132.03, 132.13. HRMS (ESI): calcd for $C_{18}H_{19}N_2O_2S$ (M+H) 327.1167; found 327.1165.

5.2.12. *N*-Benzyl-*N*-ethyl-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (18)

Colorless oil (58% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.12–1.25 (m, 3H), 3.35–3.50 (m, 2H), 4.60–4.71 (m, 3H), 4.78 (s, 1H), 7.20–7.41 (m, 6H), 7.49–7.62 (m, 2H), 7.96–8.04 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.95, 41.53, 44.82, 48.62, 120.49, 125.54, 126.42, 126.92, 127.75, 127.97, 128.43, 128.85, 129.18, 132.25, 166.07, 166.90. HRMS (ESI): calcd for $C_{18}H_{19}N_2O_2S$ (M+H) 327.1167; found 327.1165.

5.2.13. *N*,*N*-Diethyl-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (19)

Yellow oil (38% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.15 (t, J = 6.2 Hz, 3H), 1.26 (t, J = 6.2 Hz, 3H), 3.36–4.44 (m, 4H), 4.70 (s, 2H), 7.35–7.42 (m, 1H), 7.54–7.62 (m, 2H), 8.01–8.05 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.11, 14.56, 40.98, 42.01, 44.84, 120.45, 123.72, 125.50, 126.91, 132.21, 141.97, 165.90, 165.93. HRMS (ESI): calcd for $C_{13}H_{16}N_2O_2S$ (M+Na) 287.0830; found 287.0834.

5.2.14. (*S*)-2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-(1-phenylethyl)acetamide (20)

White solid (76% yield), mp 191–193 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46–1.48 (d, J = 6.9 Hz, 3H), 4.51 (s, 2H), 5.07–5.16 (q, J = 7.1 Hz, 1H), 6.72–6.75 (d, J = 6.97 Hz, 1H), 7.20–7.32 (m, 5H), 7.41–7.46 (m, 1H), 7.56–7.59 (m, 1H), 7.64–7.69 (m, 1H), 8.02–8.05 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.14, 48.03, 49.35, 120.67, 123.48, 127.05, 128.86, 132.66, 142.85, 166.30, 166.31. HRMS (ESI): calcd for $C_{17}H_{17}N_2O_2S$ (M+H) 313.1011; found 313.1002.

5.2.15. (*R*)-2-(3-Oxobenzo[d]isothiazol-2(3*H*)-yl)-*N*-(1-phenylethyl)acetamide (21)

White solid (80% yield), mp 197–199 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46–1.48 (d, J = 6.9 Hz, 3H), 4.51 (s, 2H), 5.07–5.16 (q, J = 7.4 Hz, 1H), 6.75–6.78 (d, J = 7.3 Hz, 1H), 7.20–7.32 (m, 5H), 7.41–7.46 (m, 1H), 7.56–7.59 (m, 1H), 7.64–7.69 (m, 1H), 8.02–8.05 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.16, 47.99, 49.34, 120.69, 123.52, 127.04, 128.89, 132.67, 142.91, 166.34, 166.35. HRMS (ESI): calcd for $C_{17}H_{17}N_2O_2S$ (M+H) 313.1011; found 313.1000.

5.2.16. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-phenethylacetamide (22)

White solid (0.17 g, 23% yield), mp 173–174 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.75–2.82 (t, 2H), 3.43–3.58 (q, 2H), 4.50 (s, 2H), 6.50 (s, 1H), 7.10–8.12 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 35.60, 40.90, 47.95, 120.66, 120.69, 123.51, 126.01, 126.05, 126.63, 127.08, 128.68, 128.86, 132.69, 138.58, 141.01, 165.99, 167.17. HRMS (ESI): calcd for $C_{17}H_{17}N_2O_2S$ (M+H) 313.1011; found 313.1021.

5.2.17. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-1-adamantylacetamide (23)

Yellow solid (15% yield), mp 204–205 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.80 (s, 6H), 1.93 (s, 6H), 2.0 (s, 3H), 4.4 (s, 2H), 7.30–8.00 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 28.77, 35.95, 41.07, 45.85, 51.15, 121.65, 123.50, 125.61, 131.88, 141.46, 165.27, 165.38. HRMS (ESI): calcd for $C_{19}H_{23}N_2O_2S$ (M+H) 343.1480; found 343.1483.

5.2.18. 2-(3-Oxobenzo[*d*]isothiazol-2(3*H*)-yl)-*N*-2-thiazolylacetamide (24)

Brown solid (0.27 g, 46% yield), mp 218–220 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.8 (s, 2H), 7.22 (d, 1H), 7.41–7.58 (m, 2H), 7.65 (t, 1H), 7.9 (d, 1H), 8.0 (d, 1H), 12.5 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 45.35, 113.86, 121.91, 123.26, 125.47, 125.66, 132.11, 137.79, 141.53, 157.55, 165.07, 165.85. HRMS (ESI): calcd for $C_{12}H_{19}N_3O_2S_2$ (M+Na) 314.0034; found 314.0025.

5.2.19. *N*-(2-Morpholinoethyl)-2-(3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)acetamide (25)

White solid (0.42 g, 37% yield), mp 187–189 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.2–3.4 (m, 4H), 3.2–3.25 (m, 4H), 3.5–3.6 (t, 4H), 4.82–4.89 (d, 2H), 7.3–8.03 (m, 4H), 8.90–9.00 (t, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 35.88, 42.65, 53.26, 57.30, 66.14, 125.61, 125.84, 128.38, 131.47, 132.69, 137.25, 167.11, 168.53. HRMS (ESI). calcd for $C_{15}H_{19}N_3O_3S$ (M+Na) 344.1045; found 344.1031.

5.3. Biology

5.3.1. Strains

For screening experiments, CAF2 (*C. albicans*) and RC-201, a clinical strain of *C. glabrata* (received from the Laboratory of Clinical Microbiology Lab and Immunology, Georgetown University Hospital) was used. This strain of *C. glabrata* is azole-sensitive. For further testing, *C. lusitaniae, C. guillermondii, C. tropicalis, C. parapsilosis* and *C. apicola* (clinical strains) were used. AF-294 was used to test for activity against *A. fumigatus* and, H-99 and JEC-21 strains were used to test for activity against *C. neoformans*.

5.3.2. MIC-50 determination (broth microdilution method)

The MIC-50 of *Candida* sp., *C. neoformans* and *A. fumigatus* were determined in accordance with the guidelines in CSLI standard M27-A3 and CLSI M38-A2.[16] Briefly, 1000 cells of each strain in RPMI were added to each well of the 96-well microliter-plate to a final volume of 100 μL in RPMI. Two-fold dilutions of the compounds were prepared with concentrations ranging from 0.1 to 50 μg/ml to a final volume of 100 μL in RPMI. The plates were read using a spectrophotometer at OD_{595} after 24 h for *Candida* sp., 48 h for *Aspergillus* sp. and 72 h for *Cryptococcus* sp. The MIC-50 is the lowest concentration of compound that inhibits growth to half the OD of the non-treated control.

6. Toxicity assays

The neutral red cell viability assay and the MTT assays were conducted with compound **16** at defined concentrations in human hepatoma cell lines, Huh7 and HepG2, as described by Mosmann et al.[17] and Repetto et al.,[18] respectively. The cell cytotoxicity (CC-50) concentrations were calculated for the HepG2 cell line.

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